

CLAIMS

1. A protein of fungal origin having epoxide hydrolase activity, such as is obtained in essentially pure form by extraction from cells of fungi, or by culture of host
5 cells transformed by a nucleotide sequence coding for the aforementioned fungal protein, or protein derived by substitution, suppression or addition of one or more amino acids of the aforementioned protein of fungal origin and possessing epoxide hydrolase activity.

10 2. A protein according to Claim 1, characterized in that it comprises:
– the sequence SEQ ID NO : 2,
– or any sequence derived from the sequence SEQ ID NO : 2, especially by substitution, suppression or addition of one or more amino acids, and possessing epoxide hydrolase activity, the said derived sequence preferably having a homology of
15 at least about 40% with the sequence SEQ ID NO : 2,
– or any fragment of the sequence SEQ ID NO : 2, or of a sequence derived from the latter as defined above, and possessing epoxide hydrolase activity, the said fragment preferably consisting of at least about 10 amino acids that are contiguous in the region delimited by the amino acids located in positions 1 and 339 of the sequence
20 SEQ ID NO : 2.

25 3. A protein according to Claim 1 or 2, characterized in that it corresponds to a fungal epoxide hydrolase in essentially pure form, such as is obtained by extraction and purification from cultures of cells of fungi of the *Aspergillus* species.

30 4. A protein according to one of the Claims 1 to 3, characterized in that it corresponds to the fungal epoxide hydrolase in essentially pure form represented by SEQ ID NO : 2, such as is obtained by extraction and purification from cultures of cells of strains of *Aspergillus niger* or of *Aspergillus turingensis*.

5. A protein according to Claim 1 or 2, characterized in that it corresponds to a recombinant fungal epoxide hydrolase, such as is obtained in essentially pure form by transformation of suitable host cells by means of vectors containing:

– the nucleotide sequence SEQ ID NO : 1 encoding the epoxide hydrolase represented by SEQ ID NO : 2, or any sequence derived from SEQ ID NO : 1 by degeneration of the genetic code, and encoding the epoxide hydrolase represented by SEQ ID NO : 2,

5 – or any sequence derived from the sequence SEQ ID NO : 1, especially by substitution, suppression or addition of one or more nucleotides, and coding for an enzyme possessing epoxide hydrolase activity, the said derived sequence preferably having a homology of at least about 45% with the sequence SEQ ID NO : 1,

10 – or any fragment of the sequence SEQ ID NO : 1, or of a sequence derived from the latter as defined above, and coding for an enzyme possessing epoxide hydrolase activity, the said fragment preferably consisting of at least about 20 nucleotides that are contiguous in the region delimited by the nucleotides located in positions 1 and 1197 of the sequence SEQ ID NO : 1.

15 6. A protein according to Claim 5, characterized in that it corresponds to the fungal recombinant epoxide hydrolase represented by SEQ ID NO : 2, such as is obtained by transformation of suitable host cells by means of vectors containing the nucleotide sequence SEQ ID NO : 1, or any sequence derived from SEQ ID NO : 1 by degeneration of the genetic code, and encoding the epoxide hydrolase represented by
20 SEQ ID NO : 2.

7. A nucleotide sequence encoding a protein of fungal origin with epoxide hydrolase activity such as is defined by one of the Claims 1 to 6.

25 8. A nucleotide sequence according to Claim 7, characterized in that it comprises:

– the sequence represented by SEQ ID NO : 1 encoding the epoxide hydrolase represented by SEQ ID NO : 2,

30 – or any sequence derived from the sequence SEQ ID NO : 1 by degeneration of the genetic code, and encoding the epoxide hydrolase represented by SEQ ID NO : 2,

– or any sequence derived from the sequence SEQ ID NO : 1, especially by substitution, suppression or addition of one or more nucleotides, and coding for an enzyme possessing epoxide hydrolase activity, the said derived sequence preferably having a homology of at least about 45% with the sequence SEQ ID NO : 1,

– or any fragment of the sequence SEQ ID NO : 1, or of a sequence derived from the latter as defined above, and coding for an enzyme possessing epoxide hydrolase activity, the said fragment preferably consisting of at least about 20 nucleotides that are contiguous in the region delimited by the nucleotides located in positions 1 and 1197 of the sequence SEQ ID NO : 1,

– or any complementary nucleotide sequence of the aforementioned sequences or fragments,

– or any nucleotide sequence coding for an enzyme possessing epoxide hydrolase activity, and capable of hybridization with one of the aforementioned sequences or fragments,

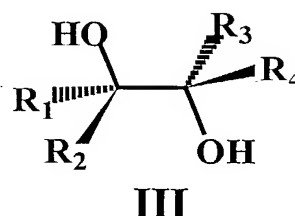
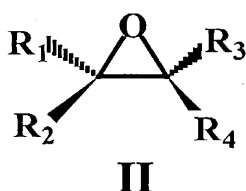
the aforementioned sequences or fragments being of single-stranded or double-stranded form.

9. A vector, especially a plasmid, containing a nucleotide sequence according to Claim 7 or 8.

10. A host cell, in particular chosen from bacteria, viruses, yeasts, fungi, plants or mammalian cells, the said host cell being transformed, especially by means of a vector according to Claim 9, in such a way that its genome contains a nucleotide sequence according to Claim 7 or 8.

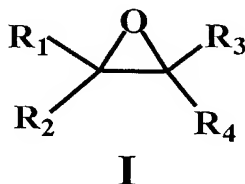
11. The use of proteins with epoxide hydrolase activity defined in one of the Claims 1 to 6, as enzymatic biocatalysts in the implementation of methods of preparation of epoxides or of enantiomerically pure vicinal diols, especially in the pharmaceutical and plant-protection field, or in the field of manufacture of specific optical materials.

12. A method of preparation of epoxides and/or of enantiomerically pure diols respectively of the following formulae (II) and (III)



in which R₁, R₂, R₃ and R₄ represent any groups, especially groups that are characteristic of pharmaceutical and plant-protection compounds, or of specific optical materials corresponding to the said epoxides or vicinal diols,

the said method comprising a stage of treatment of a mixture of diastereoisomeric epoxides, or of a chiral epoxide in racemic form, or of a prochiral epoxide of the following formula (I):



with a protein with epoxide hydrolase activity according to one of the Claims 1 to 6, or with the host cells according to Claim 10 expressing a protein with epoxide hydrolase activity according to one of the Claims 1 to 6, which leads to the production of:

- a mixture of the aforementioned compounds of formulae (II) and (III), it being possible, if necessary, for the said compounds of formulae (II) and (III) to be separated by an additional stage of purification,
- or of just the aforementioned compound of formula (III).

13. A method of preparation of a protein with recombinant epoxide hydrolase activity according to Claim 5 or 6, characterized in that it comprises a stage of transformation of host cells, preferably chosen from the bacteria, viruses, yeasts, fungi, plants or mammalian cells, with a vector according to Claim 9, and a stage of purification of the recombinant epoxide hydrolase produced by the said cells.

14. A method of preparation of a protein with epoxide hydrolase activity in essentially pure form according to Claim 3 or 4, the said method comprising:

- a stage of extraction of the enzyme from cellular cultures of fungi, such as fungi of the *Aspergillus* species, especially by crushing the fungus using a press, followed by a stage of low-speed centrifugation, recovery of the supernatant, and, if required, concentration,

— a stage of purification of the enzyme from the extract obtained in the preceding stage, especially by successive passages through columns of DEAE-Sepharose, Phenyl-Sepharose, Mono Q and Superose 12.